UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

August 26, 2013

MEMORANDUM

Subject: Efficacy Review for Hydris™
EPA Reg. No. 1677-EUR
DP Barcode: D412101

From: Marcus Rindal, Microbiologist 8.26.13
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510P)

Thru: Mark Perry, Team Leader MJP 8/26/13
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510P)

To: Michael Mendelsohn, PM 32/ Nathan Mottl
Regulatory Management Branch II
Antimicrobials Division (7510P)

Applicant: Ecolab
370 Wabash Street North
St.Paul, MN 55102-1390

FORMULATION FROM LABEL:

Active Ingredient(s)	% by wt.
Sodium Hypochlorite	0.0866%
Inert Ingredients	99.9134%
Total	100.0000%

I. BACKGROUND

The product Hydris™ is a new registration. It is a disinfectant (bactericide, virucide, fungicide) and non-food contact sanitizer for use on hard non-porous surfaces in commercial institutional, and hospitality housekeeping settings. The Hydris use dilution is generated in conjunction with the Hydris Mineral Activator Tablet and a pesticide device (three-chamber electrolytic cell) to produce the Hydris sodium hypochlorite solution generated onsite with no resale or distribution. Studies were conducted by Ecolab, Ecolab Schuman Campus, 655 Lone Oak Drive, Eagan, MN 55121-1560 and ATS Labs, 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121.

This data package contained: a letter from the registrant (dated March 25, 2013), Data Matrix (EPA Form 8570-35), a proposed product label and twenty three efficacy studies (MRID 490895-06 through -28) with a Statement of No Data Confidentiality Claims embedded in each study.

II. USE DIRECTIONS

The product, Hydris™ is intended to be used as a disinfectant and sanitizer on hard, non-porous, non-food contact surfaces including bath tubs, classroom desks, countertops, diaper changing tables, door knobs, elevators, examination tables, floors, glass, patient beds, shower stalls, toilets, urinals, and walls.

The draft label provides the following generic use directions: Spray solution onto hard, non-porous surface, thoroughly wetting surfaces. Hold sprayer 6-8 inches from the surface. Spread solution with a disposable, cotton or microfiber wipe, sponge, or cloth. Allow surface to remain wet for time indicated. No rinsing necessary.

BACTERICIDAL / DISINFECTANT: in 5 and 10 minutes at 866 ppm sodium hypochlorite (825 ppm Free Available Chlorine) against pathogenic bacteria listed on the label in the presence of 5% blood serum when solution is sprayed onto hard, non-porous surfaces.

VIRUCIDAL: in 30 seconds at 866 ppm sodium hypochlorite (825 ppm Free Available Chlorine) against the following viruses in the presence of 5% blood serum on hard, non- porous surfaces against the following organisms:

> Influenza A virus H1N1Strain (ATCC VR-1736), Norovirus (Feline Calicivirus, strain F-9 ATCC VR-782 as Surrogate), Murine Norovirus (Strain MNV-1.CW1), Rhinovirus type 37, strain 151-1 (ATCC VR-1147), Herpes Simplex Virus Type I (ATCC VR-733 Strain F), Herpes Simplex Virus Type II (ATCC VR-734, Strain G), HIV-1 (Strain HTLV-III_B).

VIRUCIDAL: in 30 seconds at 273 ppm sodium hypochlorite (260 ppm Free Available Chlorine) against the following viruses in the presence of 5% blood serum and 400 ppm hard water on hard, non-porous surfaces against the following organisms:

> Rhinovirus type 37, strain 151-1 (ATCC VR-1147), Norovirus (Feline Calicivirus, strain F-9 ATCC VR-782 as Surrogate), Influenza A virus H1N1 Strain (ATCC VR-1736),

VIRUCIDAL: in 5 minutes at 866 ppm sodium hypochlorite (825 ppm Free Available Chlorine) against the following viruses in the presence of 5% blood serum on hard, non- porous surfaces against the following organisms:

> Adenovirus Type 5 (ATCC VR-5), Hepatitus B Virus (HBV), HumaCoronavirus (ATCC

VR-740), Respiratory Syncytial Virus (RSV) (ATCC VR-26), Rotavirus (Strain WA), Vaccinia Virus (ATCC VR-119).

FUNGICIDAL: in 10 minutes at 866 ppm sodium hypochlorite 825 ppm Free Available Chlorine) according to the AOAC Fungicidal Test in the presence of 5% blood serum on hard, non-porous surfaces against *Trichophyton mentagrophytes* (TCC 9533), and *Aspergillus niger* (ATCC 6275).

III. AGENCY STANDARDS

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments: The effectiveness of disinfectants for use on hard surfaces in hospital or medical environments must be substantiated by data derived using the AOAC Use-Dilution Method (for water soluble powders and liquid products) or the AOAC Germicidal Spray Products Test (for spray products), or the AOAC Hard Surface Carrier Test. The tests require that sixty carriers must be tested with each of 3 samples, representing 3 different batches, one of which is at least 60 days old, against *Staphylococcus aureus* ATCC 6538 (for effectiveness against Gram-positive bacteria), and *Pseudomonas aeruginosa* ATCC 15442 (representative of a nosocomial pathogen), [120 carriers per sample; a total of 360 carriers] To support products labeled as "disinfectants", killing on 59 out of 60 carriers is required to provide effectiveness at the 95% confidence level. To pass performance requirements when using AOAC Hard Surface Carrier Test, tests must result in killing in 58 out of each set of 60 carriers for *Salmonella enterica* ATCC 10708 and *Staphylococcus aureus* ATCC 6538; 57 out of each set of 60 carriers for *Pseudomonas aeruginosa* ATCC 15442.

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments (Additional Bacteria): Effectiveness of disinfectants against specific bacteria other than those named in the AOAC Use-Dilution Method, AOAC Germicidal Spray Products as Disinfectants Method, AOAC Fungicidal Test, and AOAC Tuberculocidal Activity Method, must be determined by either the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products as Disinfectants Method. Ten carriers must be tested against each specific microorganism with each of 2 product samples, representing 2 different product lots. To support products labeled as "disinfectants" for specific bacteria (other than those bacteria named in the above test methods), killing of the specific microorganism on all carriers is required.

Disinfectants for Use as Fungicides (Against Pathogenic Fungi, Using a Modified AOAC Use-Dilution Method): The effectiveness of liquid disinfectants against specific pathogenic fungi must be supported by efficacy data using an appropriate test. The AOAC Use-Dilution Method may be modified to conform with the appropriate elements in the AOAC Fungicidal Test. The inoculum in the test must be modified to provide a concentration of at least 10^6 conidia per carrier. Ten carriers on each of 2 product samples representing 2 different product lots must be employed in the test. Killing of the specific pathogenic fungi on all carriers is required.

Note: As an interim policy, EPA is accepting studies with dried carrier counts that are at least 10^4 for *Trichophyton mentagrophytes*, *Aspergillus niger*, and *Candida albicans*. EPA recognizes laboratories are experiencing problems in maintaining dried carrier counts at the 10^6 level. This interim policy will be in effect until EPA determines that the laboratories are able to achieve consistent carrier counts at the 10^6 level.

Virucides: The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray disinfectants) must be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different product lots of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level.

Sanitizer Test (for inanimate, non-food contact surfaces): The effectiveness of sanitizers for non-food contact surfaces must be supported by data that show that the product will substantially reduce the numbers of test bacteria on a treated surface over those on an untreated control surface. The test surface(s) should represent the type(s) of surfaces recommended for treatment on the label, i.e., porous or non-porous. Products that are represented as "one-step sanitizers" should be tested with an appropriate organic soil load, such as 5 percent serum. Tests should be performed with each of 3 product samples, representing 3 different product lots, one of which is at least 60 days old against *Staphylococcus aureus* (ATCC 6538) and either *Klebsiella pneumoniae* (aberrant, ATCC 4352) or *Enterobacter aerogenes* (ATCC 13048 or 15038). Results must show a bacterial reduction of at least 99.9 percent over the parallel control within 5 minutes.

Supplemental Claims: An antimicrobial agent identified as a "one-step" disinfectant or as effective in the presence of organic soil must be tested for efficacy with an appropriate organic soil load, such as 5 percent serum. On a product label, the hard water tolerance level may differ with the level of antimicrobial activity (e.g., sanitizer vs. disinfectant) claimed. To establish efficacy in hard water, all microorganisms (i.e., bacteria, fungi, and viruses) claimed to be controlled must be tested by the appropriate Recommended Method at the same hard water tolerance level.

IV. Brief Description of the Data

1. MRID 490895-06 "Aqualogic Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Virus: Rhinovirus 37-260 ppm." Test Organism: Rhinovirus 37, strain 151-1 ATCC VR-1147, for product Aqualogic, Lot Numbers 051512DT-2 and 052912DT-2. Study Director Lisa Hellickson. Study conducted by Ecolab. Study completion date, Dec 19, 2012. Study ID Number 1200063.

This study was conducted using two lots of Aqualogic Lots 051512DT-2 (batch 2) and 052912DT-2 (batch 2), which were tested according to Ecolab Microbiological Services SOP Method; *Virucidal Efficacy Assay for Hard Surfaces* to determine the Virucidal efficacy against Rhinovirus Type 37-260 ppm (strain 151-1 ATCC VR-1147), with a 5% fetal bovine serum organic soil load, after 30 seconds exposure at ambient temperature. Testing followed GLP Protocol Study No. 1200063 (copy provided). The host cell line was HeLa cells (ATCC CRL-2)

which were prepared from the third or greater transfer to 24 well assay plates, incubated at 35±2°C at 5±2% CO_2 for two days. Test medium used to maintain the cell cultures was Minimum Essential Medium, Eagle (EMEM) test medium, supplemented with 50-100 IU penicillin and 50-t00 µg/mL streptomycin. The two lots of test substance were diluted to 260 ppm free available chlorine in sterile 400 ppm synthetic hard water. Lot 051512DT-2 dilution 97.85 g test substance plus 252.15 g diluent and Lot 052912DT-2 dilution 100.10 g test substance plus 249.9t g diluent to achieve 260 ppm available chlorine. Several vials of stock virus culture of Rhinovirus Type 37-260 ppm was thawed and pooled on the day of the test and since 5% Fetal Bovine Serum (FBS) is present in the virus stock, no additional soil load was added. The bottom of t00×15 mm glass Petri dishes were inoculated with 0.2 mL of prepared virus/soil mixture and dried for 30-33 minutes in a biological safety cabinet. Each batch of use-solution was dispensed into one glass Petri dish with a dried virus film by spraying at a distance of 6 to 8 inches using 3 trigger pulls. The test was conducted at ambient (15°-30°C) temperature. Near the end of the 30 second exposure time, the dried film was scraped from the surface of the dish with a cell scraper, and 0.1 mL of the virus/test mixture was pipetted into prepared sephacryl columns (0.t mL per column – five columns used per batch) for neutralization. The columns were centrifuged for 2 minutes at 700×g after which the supernatant was inoculated into assay plates seeded with the test cell culture (0.1 mL per well) and serially diluted to inoculate assay plates. Four assay wells were inoculated per dilution prepared and were incubated at 35±2°C in a humidified atmosphere at 5±2% CO_2 for 7 days. Controls included those for dried virus film recovery, virus stock titer confirmation, cytotoxicity, neutralization and cell viability.
Note: Protocol deviations/amendments reported in this study were reviewed and found to be acceptable.

2. MRID 490895-07 "Aqualogic Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Virus: Rhinovirus 37-660 ppm." Test Organism: Rhinovirus 37, strain 151-1 ATCC VR-1147, for product Aqualogic, Lot Numbers 051512DT-2 and 052912DT-2. Study Director Lisa Hellickson. Study conducted by Ecolab. **Study completion date, Dec 19, 2012. Study ID Number 1200062.**

This study was conducted using two lots of Aqualogic Lots 05t512DT-2 (batch 2) and 052912DT-2 (batch 2), which were tested according to Ecolab Microbiological Services SOP Method; *Virucidal Efficacy Assay for Hard Surfaces* to determine the Virucidal efficacy against Rhinovirus Type 37-660 ppm (strain 151-1 ATCC VR-1147), with a 5% fetal bovine serum organic soil load, after 30 seconds exposure at ambient temperature. Testing followed GLP Protocol Study No. 1200062 (copy provided). The two lots of test substance were diluted to the lower certified limit of 660 ppm available chlorine in sterile lab purified water. Lot 051512DT-2 dilution 248.00 g test substance plus 102.00 g diluent and Lot 052912DT-2 dilution 253.76 g test substance plus 96.25 g diluent to achieve 660 ppm available chlorine. The host cell line was HeLa cells (ATCC CCL-2) which were prepared from the third or greater transfer to 24 well assay plates, incubated at 35±2°C at 5±2% CO_2 for two days. Test medium used to maintain the cell cultures was Minimum Essential Medium, Eagle (EMEM) test medium, supplemented with 50-100 IU penicillin and 50-100 µg/mL streptomycin. Several vials of stock virus culture of Rhinovirus Type 37-660 ppm was thawed and pooled on the day of the test and since 5% Fetal Bovine Serum (FBS) is present in the virus stock, no additional soil load was added. The bottom of t00 x 15 mm glass petri dishes were inoculated with 0.2 mL of prepared virus/soil mixture and dried for 32-35 minutes in a biological safety cabinet. Each batch of use-solution was dispensed into one glass petri dish with a dried virus film by spraying at a distance of 6 to 8 inches using 3 trigger pulls. The test was conducted at ambient (15° - 30° C) temperature. At the end of the 30 second exposure time, the dried film was scraped from the surface of the dish

with a cell scraper, and 0.t mL of the virus/test mixture was pipetted into prepared sephacryl columns (0.t mL per column – five columns used per batch) for neutralization. The columns were centrifuged for 2 minutes at 700×g after which the supernatant was inoculated into assay plates seeded with the test cell culture (0.1 mL per well) and serially diluted in test medium to inoculate assay plates. Four assay wells were inoculated per dilution prepared and were incubated at 35±2°C in a humidified atmosphere at 5±2% CO_2 for 7 days. Controls included those for dried virus film recovery, virus stock titer confirmation, cytotoxicity, neutralization and cell viability.
Note: Protocol deviations/amendments reported in this study were reviewed and found to be acceptable.

3. MRID 490895-08 "Aqualogic Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Virus: Feline Calicivirus as a Surrogate for Norovirus -660 ppm". Test Organism: Feline Calicivirus, strain F-9 ATCC VR-782, for product Aqualogic, Lot Numbers 051512DT-2, 051512DT-3 and 052912DT-1. Study Director Lisa Hellickson. Study conducted by Ecolab. Study completion date, Dec 20, 2012. Study ID Number 1200066.

This study was conducted using two lots of Aqualogic (Lots 051512DT and 0529t2DT), which were tested according to Ecolab Microbiological Services SOP Method; *Virucidal Efficacy Assay for Hard Surfaces* to determine the Virucidal efficacy against Feline Calicivirus (strain F-9 ATCC VR-782), with a 5% fetal bovine serum organic soil load, after 30 seconds exposure at ambient temperature. Testing followed GLP Protocol Study No. 1200066 (copy provided). Lot 051512DT-3 (batch 3 tested 07/17/12) dilution 246.96 g test substance plus 103.05 g diluent, Lot 0529t2DT-1 (batch 1 tested 07/17/12) dilution 253.75 g test substance plus 96.26 g diluent, Lot 0515t2DT-2 (batch 2 tested 08/03/12) dilution 215.12 g test substance plus 84.85 g diluent and Lot 051512DT-2 (batch 2 tested 08/09/12) dilution 213.95 g test substance plus 86.04 g diluent to achieve 660 ppm available chlorine. The host cell line was CRFK cells (ATCC CCL-94) which was prepared from the third or greater transfer to 24 well assay plates, incubated at 35±2°C at 5±2% CO_2 for two days. Test medium used to maintain the cell cultures was Minimum Essential Medium, Eagle (EMEM) test medium, supplemented with 50-100 IU penicillin and 50-100 µg/mL streptomycin. The two lots of test substance were diluted to the lower certified limit of 660 ppm available chlorine in sterile laboratory purified water. Several vials of stock virus culture of Feline Calicivirus was thawed and pooled on the day of the test and since 5% horse serum is present in the virus stock, no additional soil load was added. The bottom of 100 x 15 mm glass Petri dishes were inoculated with 0.2 mL of prepared virus/soil mixture and dried in a biological safety cabinet for 38 minutes on 07/17/12, 35-36 minutes on 08/03/12 and 38 minutes on 08/09/12. Each batch of use-solution was dispensed into one glass petri dish with a dried virus film by spraying at a distance of 6 to 8 inches using 3 trigger pulls at ambient (15° - 30° C) temperature. At the end of the 30 second exposure time, the dried film was scraped from the surface of the dish with a cell scraper, and 0.1 mL of the virus/test mixture was pipetted into prepared sephacryl columns (0.t mL per column – five columns used per batch) for neutralization. The columns were centrifuged for 2 minutes at 700 x g after which the supernatant was inoculated into assay plates seeded with the test cell culture (0.1 mL per well) and serially diluted in test medium to inoculate assay plates. Four assay wells were inoculated per dilution prepared and were incubated at 35±2°C in a humidified atmosphere at 5±2% CO_2 for 7-10 days. Controls included those for dried virus film recovery, virus stock titer confirmation, cytotoxicity, neutralization and cell viability.
Note: Protocol deviations/amendments reported in this study were reviewed and found to be acceptable.

4. MRID 490895-09 "Aqualogic Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Virus: Feline Calicivirus as a Surrogate for Norovirus -260 ppm". Test Organism: Feline Calicivirus, strain F-9 ATCC VR-782, for product Aqualogic, Lot Numbers 051512DT-2 and 052912DT-2. Study Director Lisa Hellickson. Study conducted by Ecolab. Study completion date, Dec 20, 2012. Study ID Number 1200067.

This study was conducted using two lots of Aqualogic, Lots 0515t2DT-2 (batch 2) and 052912DT-2 (batch 2), which were tested according to Ecolab Microbiological Services SOP Method; *Virucidal Efficacy Assay for Hard Surfaces* to determine the Virucidal efficacy against Feline Calicivirus (strain F-9 ATCC VR-782), with a 5% fetal bovine serum organic soil load, after 30 seconds exposure at ambient temperature. Testing followed GLP Protocol Study No. t200067 (copy provided). The two lots of test substance were diluted to the lower certified limit of 260 ppm available chlorine in 400 ppm sterile synthetic hard water. Lot 05t512DT-2 dilution 84.85 g test substance plus 2t5.12 g diluent and Lot 052912DT-2 dilution 87.05 g test substance plus 212.95 g diluent to achieve 260 ppm available chlorine. The host cell line was CRFK cells (ATCC CCL-94) which was prepared from the third or greater transfer to 24 well assay plates, incubated at 35±2°C at 5±2% CO_2 for two days. Test medium used to maintain the cell cultures was Minimum Essential Medium, Eagle (EMEM) test medium, supplemented with 50-100 IU penicillin and 50-100 µg/mL streptomycin. Several vials of stock virus culture of Feline Calicivirus were thawed and pooled on the day of the test and since 5% horse serum is present in the virus stock, no additional soil load was added. The bottom of t00 x t5 mm glass petri dishes were inoculated with 0.2 mL of prepared virus/soil mixture and dried in a biological safety cabinet for 35 minutes. Each batch of use-solution was dispensed into one glass petri dish with a dried virus film by spraying at a distance of 6 to 8 inches using 3 trigger pulls at ambient (t5° - 30° C) temperature. At the end of the 30 second exposure time, the dried film was scraped from the surface of the dish with a cell scraper, and 0.1 mL of the virus/test mixture was pipetted into prepared sephacryl columns (0.t mL per column – five columns used per batch) for neutralization. The columns were centrifuged for 2 minutes at 700 x g after which the supernatant was inoculated into assay plates seeded with the test cell culture (0.1 mL per well) and serially diluted in test medium to inoculate assay plates. Four assay wells were inoculated per dilution prepared and were incubated at 35±2°C in a humidified atmosphere at 5±2% CO_2 for 7-t0 days. Controls included those for dried virus film recovery, virus stock titer confirmation, cytotoxicity, neutralization and cell viability.
Note: Protocol deviations/amendments reported in this study were reviewed and found to be acceptable.

5. MRID 490895-10 "Aqualogic Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Virus: Herpes Simplex Type 1." Test Organism: Rhinovirus 37, strain F (1) ATCC VR-733, for product Aqualogic, Lot Numbers 051512DT-2 and 052912DT-2. Study Director Lisa Hellickson. Study conducted by Ecolab. Study completion date, Dec 12, 2012. Study ID Number 1200064.

This study was conducted using two lots of Aqualogic Lots 05t5t2DT-2 (batch 2) and 0529t2DT-2 (batch 2), which were tested according to Ecolab Microbiological Services SOP Method; *Virucidal Efficacy Assay for Hard Surfaces* to determine the Virucidal efficacy against Herpes Simplex Type 1 (strain F (t) ATCC VR-733), with a 5% fetal bovine serum organic soil load, after 30 seconds exposure at ambient temperature. Testing followed GLP Protocol Study No. 1200064 (copy provided). The host cell line of Vero Cells (ATCC CLL-8t) was prepared from the third or greater transfer to 24 well assay plates, incubated at 35±2°C at 5±2% CO_2 for two days. Test medium used to maintain the cell cultures was Minimum Essential Medium,

Eagle (EMEM) test medium, supplemented with 50-100 IU penicillin and 50-100 µg/mL streptomycin. The two lots of test substance were diluted to the lower certified limit of 660 ppm available chlorine in sterile Milli-Q water. Lot 051512DT-2 dilution 314.13 g test substance plus 135.85 g diluent and Lot 052912DT-2 dilution 327.30 g test substance plus 122.66 g diluent to achieve 660 ppm available chlorine. Several vials of stock virus culture of HSV Type 1 were thawed and pooled on the day of the test and since 5% Fetal Bovine Serum (FBS) is present in the virus stock, no additional soil load was added. The bottom of 100 x 15 mm glass Petri dishes were inoculated with 0.2 mL of prepared virus/soil mixture and dried for 40-43 minutes in a biological safety cabinet. Each batch of use-solution was dispensed into one glass Petri dish with a dried virus film by spraying at a distance of 6 to 8 inches using 3 trigger pulls. The test was conducted at ambient (15° - 30° C) temperature. At the end of the 30 second exposure time, the dried film was scraped from the surface of the dish with a cell scraper, and 0.1 mL of the virus/test mixture was pipetted into prepared sephacryl columns (0.1 mL per column – five columns used per batch) for neutralization. The columns were centrifuged for 2 minutes at 700 x g after which the supernatant was inoculated into assay plates seeded with the test cell culture (0.1 mL per well) and serially diluted in test medium to inoculate assay plates. Four assay wells were inoculated per dilution prepared and were incubated at 35±2°C in a humidified atmosphere at 5±2% CO_2 for 7 to 10 days. Controls included those for dried virus film recovery, virus stock titer confirmation, cytotoxicity, neutralization and cell viability.
Note: Protocol deviations/amendments reported in this study were reviewed and found to be acceptable.

6. MRID 490895-11 "Aqualogic Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Virus: Influenza A Virus -260 ppm." Test Organism: Influenza A Virus H1N1 strain A/Virginia/ATCC1/2009 ATCC VR-1736, for product Aqualogic, Lot Numbers 051512DT-2 and 052912DT-3. Study Director Lisa Hellickson. Study conducted by Ecolab. Study completion date, Dec 12, 2012. Study ID Number 1200061.

This study was conducted using two lots of Aqualogic Lots 051512DT-2 (batch 2) and 052912DT-3 (batch 3), which were tested according to Ecolab Microbiological Services SOP Method; *Virucidal Efficacy Assay for Hard Surfaces* to determine the Virucidal efficacy against Influenza A Virus (H1N1 strain A/Virginia/ATCC1/2009 ATCC VR-1736), with a 5% fetal bovine serum organic soil load, after 30 seconds exposure at ambient temperature. Testing followed GLP Protocol Study No. 1200061 (copy provided). Assay plates seeded with Rhesus Monkey Kidney cells (RMK) were purchased from ViroMed Labs and were incubated at 35±2°C at 5±2% CO_2. Test medium used to maintain the cell cultures was Minimum Essential Medium, Eagle (EMEM) test medium, supplemented with 100 units/mL penicillin, 10 µg/mL gentamicin, 2.5 µg/mL fungizone, 10 mM Hepes, SV% & SV40 antisera. The two lots of test substance were diluted to 260 ppm free available chlorine in sterile 400 ppm synthetic hard water. Lot 051512DT-2 dilution 85.58 g test substance plus 215.42 g diluent and Lot 052912DT-3 dilution 86.85 g test substance plus 213.13 g diluent to achieve 260 ppm available chlorine. Two vials of stock virus culture of Influenza A Virus were thawed and pooled on the day of the test. Fetal Bovine Serum (FBS) was added to obtain a 5% soil load (0.1 mL FBS, 1.9 mL pooled virus). The bottom of 100 x 15 mm glass Petri dishes were inoculated with 0.2 mL of prepared virus/soil mixture and dried for 32-35 minutes in a biological safety cabinet. Each batch of use-solution was dispensed into one glass Petri dish with a dried virus film by spraying at a distance of 6 to 8 inches using 3 trigger pulls at ambient (15°-30°C) temperature. Near the end of the 30 second exposure time, the dried film was scraped from the surface of the dish with a cell scraper, and 0.1 mL of the virus/test mixture was pipetted into prepared sephacryl columns (0.1 mL per column – five columns used per batch) for neutralization. The columns were centrifuged

for 2 minutes at 700 x g after which the supernatant was inoculated into assay plates seeded with the test cell culture (0.1 mL per well) and serially diluted to inoculate assay plates. Four assay wells were inoculated per dilution prepared and were incubated at 35±2°C in a humidified atmosphere at 5±2% CO_2 for 7-10 days. Controls included those for dried virus film recovery, virus stock titer confirmation, cytotoxicity, neutralization and cell viability.
Note: Protocol deviations/amendments reported in this study were reviewed and found to be acceptable.

7. MRID 490895-12 "Aqualogic Germicidal Spray Hospital Disinfection Efficacy" for the product Aqualogic. Study director is Lisa Hellickson. Study conducted by Ecolab. Study completion date – December 17, 2012. Project Number 1200052.

This study was conducted against *Staphylococcus aureus* (ATCC 6538) *Pseudomonas aeruginosa* (ATCC 15442) and *Salmonella enterica* (ATCC t0708). Three lots (051t2DT, 052912DT, 050112DT (≥60 days old)) of the product, Aqualogic, was tested using Ecolab Microbiological Services SOP Method MS010-20 (copy provided). The Aqualogic test substance was diluted to the lower certified limit in sterile laboratory purified or Milli-Q water to yield 660 ppm available chlorine as follows:

Lot 515t2DT-1; 1054.33g + 445.68 g diluent = 660 ppm available chlorine
Lot 529t2DT-t; 1077.96g + 422.05g diluent = 660 ppm available chlorine
Lot 50112DT-t; 1072.12g + 427.85g diluent = 660 ppm available chlorine
Lot 50112DT-2; 431.59g + 168.40g diluent = 660 ppm available chlorine

As per protocol, the test organisms were prepared by inoculation from stock cultures and transferred greater than 3 times but less than or equal to t5 times in a suitable growth medium prior to being used in the test. Transfers were performed daily, but the last transfer prior to the conduct of the test were 24 ± 4 hour transfers. The culture used in the test was grown in 20 mL of culture medium, vortexed, and allowed to settle for 10 minutes. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Sixty glass slide carriers were inoculated with 0.0t mL of a suspension of test organism. The carriers were dried for 30 to 40 minutes at 35±2°C. Each carrier was sprayed with the test product until thoroughly wet at a distance of 6-8 inches from the carrier surface. Each carrier remained in contact with the product for 5 minutes at ambient temperature (t5-30°C). Following exposure, individual carriers were transferred to Letheen Broth containing 5% sodium thiosulfate to neutralize. All subcultures were incubated for 48±4 hours at 35±2°C. Following incubation, the subcultures were examined for growth. Controls included those for purity, sterility, viability, neutralization confirmation, bacteriostasis, and carrier population.
Note: Protocol deviations/amendments reported in this study report were reviewed and found to be acceptable.

8. MRID 490895-13 "Aqualogic Non-Food Contact Surface Sanitizing Efficacy 4 Minutes Exposure Time" for the product Aqualogic. Study director is Lisa Hellickson. Study conducted by Ecolab. Study completion date – May 18, 2012. Project Number 120-054.

This study was conducted against *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048). Three lots (051t2DT, 052912DT, 050tt2DT (≥60 days old)) of the product, Aqualogic, was tested using Ecolab Microbiological Services SOP Method MS016-23 (copy provided). The Aqualogic test substance was diluted to the lower certified limit in 250 ppm sterile synthetic hard water to yield 260 ppm available chlorine as follows:

Lot 515t2DT-t; 55.96g + 144.02g diluent = 260 ppm available chlorine

Lot 51512DT-2; 56.32g + 143.69g diluent = 260 ppm available chlorine
Lot 52912DT-2; 56.81g + 143.91g diluent = 260 ppm available chlorine
Lot 50112DT-1; 56.09g + 143.68g diluent = 260 ppm available chlorine

As per protocol, the test organisms were prepared by inoculation from stock cultures and transferred greater than 3 times but less than or equal to 15 times in a suitable growth medium prior to being used in the test. Transfers were performed daily, but the last transfer prior to the conduct of the test were 24 ± 4 hour transfers. The culture used in the test was grown in 10 mL of culture medium, vortexed, and allowed to settle for 15 minutes. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Five (5) 1 inch x 1 inch stainless steel carriers were inoculated with 0.01 mL of a suspension of test organism. The carriers were dried for 30 to 40 minutes at 35±2°C. The dried, inoculated carriers were aseptically transferred to sterile jars-one carrier per jar. 5 mL of each lot of Aqualogic use-solution was dispensed onto one inoculated and dried carrier at a time. Each carrier remained in contact with the product for 3 minutes for *E. aerogenes* and 4 minutes for *S. aureus* at ambient temperature (15-30°C). At the end of the exposure time, 20 mL of 2X D/E Broth was added to the jar. Each jar was then rotated on an even plane approximately 50 rotations. 1.0 mL and 0.1 mL of the neutralizer solution was plated in duplicate from each jar and pour plated with Tryptone Glucose Extract Agar. The recovery medium plates were incubated at 35 ± 2°C for 48 ± 4 hours for *S. aureus* or 30 ± 2°C for 48 ± 4 hours for *E. aerogenes*. Following incubation, the subcultures were examined for growth. Controls included those for purity, sterility, viability, neutralization confirmation, bacteriostasis, and carrier population.

Note: Protocol deviations/amendments reported in this study report were reviewed and found to be acceptable.

9. MRID 490895-14 "Aqualogic Non-Food Contact Surface Sanitizing Efficacy 1 Minute Exposure Time" for the product Aqualogic. Study director is Lisa Hellickson. Study conducted by Ecolab. Study completion date – December 17, 2012. Project Number 1200053.

This study was conducted against *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048). Three lots (051512DT, 052912DT, 050112DT (≥60 days old)) of the product, Aqualogic, was tested using Ecolab Microbiological Services SOP Method MS016-23 (copy provided). The Aqualogic test substance was diluted to the lower certified limit in sterile laboratory purified or Milli-Q water to yield 660 ppm available chlorine as follows:

Lot 51512DT-1; 141.88g + 58.11 g diluent = 660 ppm available chlorine
Lot 51512DT-1; 142.80g + 57.20 g diluent = 660 ppm available chlorine
Lot 52912DT-2; 144.02g + 55.95g diluent = 660 ppm available chlorine
Lot 50112DT-1; 142.19g + 57.82g diluent = 660 ppm available chlorine

As per protocol, the test organisms were prepared by inoculation from stock cultures and transferred greater than 3 times but less than or equal to 15 times in a suitable growth medium prior to being used in the test. Transfers were performed daily, but the last transfer prior to the conduct of the test were 24 ± 4 hour transfers. The culture used in the test was grown in 10 mL of culture medium, vortexed, and allowed to settle for 15 minutes. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Five (5) 1 inch x 1 inch stainless steel carriers were inoculated with 0.02 mL of a suspension of test organism. The carriers were dried for 35 minutes at 35±2°C. The dried, inoculated carriers were aseptically transferred to sterile jars-one carrier per jar. 5 mL of each lot of Aqualogic use-solution was dispensed onto one inoculated and dried carrier at a time. Each carrier remained in contact with the product for 1 minute at ambient temperature (15-30°C). At the end of the exposure time, 20 mL of 2X D/E Broth was added to the jar. Each jar was then rotated on an even plane approximately 50 rotations. 1.0 mL and 0.1 mL of the neutralizer solution was plated in duplicate from each jar

and pour plated with Tryptone Glucose Extract Agar. The recovery medium plates were incubated at 35 ± 2°C for 48 ± 4 hours for *S. aureus* or 30 ± 2°C for 48 ± 4 hours for *E. aerogenes*. Following incubation, the subcultures were examined for growth. Controls included those for purity, sterility, viability, neutralization confirmation, bacteriostasis, and carrier population.
Note: Protocol deviations/amendments reported in this study report were reviewed and found to be acceptable.

10. MRID 490895-15 "Aqualogic Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Virus: Influenza A Virus -660 ppm." Test Organism: Influenza A Virus H1N1 strain A/Virginia/ATCC1/2009 ATCC VR-1736, for product Aqualogic, Lot Numbers 051512DT-2 and 052912DT-3. Study Director Lisa Hellickson. Study conducted by Ecolab. Study completion date, Dec 18, 2012. Study ID Number 1200060.

This study was conducted using two lots of Aqualogic Lots 051512DT-2 (batch 2) and 052912DT-3 (batch 3), which were tested according to Ecolab Microbiological Services SOP Method; *Virucidal Efficacy Assay for Hard Surfaces* to determine the Virucidal efficacy against Influenza A Virus (H1N1 strain A/Virginia/ATCC1/2009 ATCC VR-1736), with a 5% fetal bovine serum organic soil load, after 30 seconds exposure at ambient temperature. Testing followed GLP Protocol Study No. 1200060 (copy provided). The two lots of test substance were diluted to 660 ppm free available chlorine in sterile laboratory purified water. Lot 051512DT-2 dilution 214.41 g test substance plus 85.57 g diluent and Lot 052912DT-3 dilution 220.17 g test substance plus 79.85 g diluent to achieve 660 ppm available chlorine. Assay plates seeded with Rhesus Monkey Kidney cells (RMK) were purchased from ViroMed Labs and were incubated at 35±2°C at 5±2% CO_2. Test medium used to maintain the cell cultures was Minimum Essential Medium, Eagle (EMEM) test medium, supplemented with 100 units/mL penicillin, 10 µg/mL gentamicin, 2.5 µg/mL fungizone, 10 mM Hepes, SV5 & SV40 antisera. Two vials of stock virus culture of Influenza A Virus were thawed and pooled on the day of the test. Fetal Bovine Serum (FBS) was added to obtain a 5% soil load (0.1 mL FBS, 1.9 mL pooled virus). The bottom of 100 x 15 mm glass Petri dishes were inoculated with 0.2 mL of prepared virus/soil mixture and dried for 34-36 minutes in a biological safety cabinet. Each batch of use-solution was dispensed into one glass Petri dish with a dried virus film by spraying at a distance of 6 to 8 inches using 3 trigger pulls at ambient (15°-30°C) temperature. Near the end of the 30 second exposure time, the dried film was scraped from the surface of the dish with a cell scraper, and 0.1 mL of the virus/test mixture was pipetted into prepared sephacryl columns (0.1 mL per column – five columns used per batch) for neutralization. The columns were centrifuged for 2 minutes at 700 x g after which the supernatant was inoculated into assay plates seeded with the test cell culture (0.1 mL per well) and serially diluted to inoculate assay plates. Four assay wells were inoculated per dilution prepared and were incubated at 35±2°C in a humidified atmosphere at 5±2% CO_2 for 7-10 days. Controls included those for dried virus film recovery, virus stock titer confirmation, cytotoxicity, neutralization and cell viability.
Note: Protocol deviations/amendments reported in this study were reviewed and found to be acceptable.

11. MRID 490895-16 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces", Virus: Human Immunodeficiency virus type 1, for product Aqualogic, Batch 073112-CL1 and Batch 080112-CL1. Study Director Shanen Conway, B.S., Study completion date, Oct. 8, 2012. Study conducted by ATS Labs. Project Number A13930.

This study was conducted using two batches of test substance "Aqualogic", Batch 073112-CL1 and Batch 080112-CL1, which were tested against Human Immunodeficiency virus type 1, Strain HTLV-III_B (obtained from Advanced Biotechnologies, Inc.) with a 5% fetal bovine serum organic soil load. Testing followed Protocol Number ECO01072512.HIV (copy provided). The host cell line, MT-2 cells (human T-cell leukemia cells obtained through the AIDS Research and Reference Reagent Program) was maintained and used in tissue culture at 36-38°C in a humidified atmosphere at 5-7% CO_2. Test medium used to maintain the cell cultures was RPMI-1640 supplemented with 15% (v/v) heat-activated fetal bovine serum (FBS) and with 2.0 mM L-glutamine and 50 µg/mL gentamicin. The two batches of test substance were diluted to the lower limit of 660 ppm available chlorine in sterile deionized water. Batch 073112-CL1 dilution 356.99 g test substance plus 143.01 g diluent and Batch 080112-CL1 dilution 352.78 g test substance plus 147.22 g diluent to achieve 660 ppm available chlorine. On the day use, an aliquot of stock virus HIV type 1 (strain HTLV-III_B) was thawed and adjusted to contain 5% FBS as the organic soil load. The bottoms of 100 x 15 mm glass Petri dishes were inoculated with 0.2 mL of prepared virus/soil mixture and dried at 21.0°C at 44.8% relative humidity for 20 minutes (until visibly dry). Each batch of test substance was dispensed into one glass Petri dish with a dried virus film by spraying at a distance of 6 to 8 inches using 3 trigger pulls. The test was conducted at room temperature (21.0°C). Near the end of the 30 second exposure time, the dried films were scraped with a cell scraper, and at the 30 second exposure time the virus-test substance mixtures were immediately passed through prepared individual Sephadex columns utilizing the syringe plungers to detoxify the mixtures. The filtrates (10^{-1} dilution) were then titered by 10-fold serial dilution. The MT-2 cells in multiwell culture dishes were inoculated in quadruplicate with 0.2mL of the dilutions from the test and control groups and were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 14 days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus film recovery, cytotoxicity, neutralization and cell viability.
Note: Protocol deviations/amendments reported in this study were reviewed and found to be acceptable.

12. MRID 490895-17 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces", Virus: Rotavirus (Strain WA), for product Aqualogic, Batch 073112-CL1 and Batch 080112-CL1. Study Director Shanen Conway, B.S., Study completion date, Oct. 29, 2012. Study conducted by ATS Labs. Project Number A13928.

This study was conducted using two batches of test substance "Aqualogic", Batch 073112-CL1 and Batch 080112-CL1, which were tested against Rotavirus, Strain WA (obtained from University of Ottawa, Ontario, Canada) with a 5% fetal bovine serum organic soil load. Testing followed Protocol Number ECO01072512.ROT (copy provided). The host cell line, MA-104 cells (Rhesus monkey kidney cells, obtained from Diagnostic Hybrids, Inc.) was maintained and used in tissue culture at 36-38°C in a humidified atmosphere at 5-7% CO_2. Test medium used to maintain the cell cultures was Minimum Essential Medium (MEM) supplemented with 10 µg/mL gentamicin, 100 units/mL penicillin, 2.5 µg/mL amphotericin B, 0.5 µg/mL trypsin and 2.0 mM L-glutamine. The two batches of test substance were diluted to the lower limit of 660 ppm available chlorine in sterile deionized water. Test date 8/24/12, Batch 073112-CL1 dilution 357.38 g test substance plus 142.62 g diluent and Batch 080112-CL1 dilution 352.41 g test substance plus 147.59 g diluent to achieve 660 ppm available chlorine. Test date 10/04/12, Batch 073112-CL1 dilution 358.15 g test substance plus 141.85 g diluent to achieve 660 ppm available chlorine. On the day use, an aliquot of stock virus Rotavirus, Strain WA was thawed and adjusted to contain 5% FBS as the organic soil load. Test date 8/24/12, the bottoms of three 100 x 15 mm glass Petri dishes were inoculated with 0.2 mL of prepared virus/soil mixture

and dried at 20.0°C at 50% relative humidity for 20 minutes (until visibly dry). Test date 10/04/12, the bottoms of two 100 x 15 mm glass Petri dishes were inoculated with 0.2 mL of prepared virus/soil mixture and dried at 20.0°C at 40% relative humidity for 20 minutes (until visibly dry). On each day of testing, the carriers were sprayed at a distance of 6 to 8 inches using 3 trigger pulls and were exposed for 5 minutes at room temperature (20.0°C). Near the end of the exposure time, the dried films were individually scraped with a cell scraper, and at the end of the exposure time the virus-test substance mixtures were immediately passed through prepared individual Sephadex columns utilizing the syringe plungers to detoxify the mixtures. The filtrates (10^{-1} dilution) were then titered by 10-fold serial dilution. The MA-104 cells in multiwell culture dishes were inoculated in quadruplicate with 0.1mL of the dilutions from the test and control groups and were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus film recovery, cytotoxicity, neutralization and for viability.
Note: Protocol deviations/amendments reported in this study were reviewed and found to be acceptable.

13. MRID 490895-18 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces", Virus: Vaccina Virus, for product Aqualogic, Batch 073112-CL1 and Batch 080112-CL1. Study Director Shanen Conway, B.S., Study completion date, Sept. 26, 2012, Study Conducted by ATS Labs. Project Number A13927.

This study was conducted using two batches of test substance "Aqualogic", Batch 073112-CL1 and Batch 080112-CL1, which were tested against Vaccina virus, Strain WR (ATCC VR-119) with a 5% fetal bovine serum organic soil load. Testing followed Protocol Number ECO01072512.VAC (copy provided). The host cell line, Vero cells (ATCC CCL-81) were maintained and used in tissue culture at 36-38°C in a humidified atmosphere at 5-7% CO_2. Test medium used to maintain the cell cultures was Minimum Essential Medium (MEM) supplemented with 5% (v/v) heat-activated fetal bovine serum (FBS), 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. The two batches of test substance were diluted to the lower limit of 660 ppm available chlorine in sterile deionized water. Batch 073112-CL1 dilution 357.38 g test substance plus 142.62 g diluent and Batch 080112-CL1 dilution 352.41 g test substance plus 147.59 g diluent to achieve 660 ppm available chlorine. On the day of use, an aliquot of stock Vaccina virus, Strain WR was thawed and adjusted to contain 5% FBS as the organic soil load. The bottoms of three separate 100 x 15 mm glass petri dishes were inoculated with 0.2 mL of prepared virus/soil mixture and dried at 20.0°C at 50% relative humidity for 20 minutes (until visibly dry). Each batch of test substance was dispensed into one glass petri dish with a dried virus film by spraying at a distance of 6 to 8 inches using 3 trigger pulls at room temperature (20.0°C). Near the end of the 5 minute exposure time, the dried films were scraped with a cell scraper and at the end of the exposure time the virus-test substance mixtures were immediately passed through prepared individual Sephadex columns utilizing the syringe plungers to detoxify the mixtures. The filtrates (10^{-1} dilution) were then titered by 10-fold serial dilution. The Vero cells in multiwell culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions from the test and control groups and were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus film recovery, cytotoxicity, neutralization and cell viability.
Note: Protocol deviations/amendments reported in this study were reviewed and found to be acceptable

14. MRID 490895-19 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces", Virus: Respiratory syncytial (RSV) virus, for product Aqualogic, Batch 073112-CL1 and Batch 080112-CL1. Study Director Mary J. Miller, M.T., Study completion date, Sept. 21, 2012. Study Conducted by ATS Labs. Project Number A13925.

This study was conducted using two batches of test substance "Aqualogic", Batch 073112-CL1 and Batch 080112-CL1, which were tested against Respiratory syncytial (RSV) virus (ATCC VR-26, Strain Long) with a 5% fetal bovine serum organic soil load. Testing followed Protocol Number ECO01072512.RSV (copy provided). The host cell line, Hep-2 (human larynx carcinoma) cells, obtained from ViroMed Labs, were maintained and used in tissue culture at 36-38°C in a humidified atmosphere at 5-7% CO_2. Test medium used to maintain the cell cultures was Eagle's Minimum Essential Medium (E-MEM) supplemented with 2% (v/v) heat-activated fetal bovine serum (FBS), 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL Fungizone, 10 mM HEPES, 10 µg/mL vancomycin and 2 mM L-glutamine. The two batches of test substance were diluted to the lower limit of 660 ppm available chlorine in sterile deionized water. Batch 073112-CL1 dilution 357.38 g test substance plus 142.62 g diluent and Batch 080112-CL1 dilution 352.41 g test substance plus 147.59 g diluent to achieve 660 ppm available chlorine. On the day of use, an aliquot of stock RSV virus, was thawed and adjusted to contain 5% FBS as the organic soil load. The bottoms of three separate 100 x 15 mm glass Petri dishes were inoculated with 0.2 mL of prepared virus/soil mixture and dried at 20.0°C at 50% relative humidity for 20 minutes (until visibly dry). Each batch of test substance was dispensed into one glass Petri dish with a dried virus film by spraying at a distance of 6 to 8 inches using 3 trigger pulls at room temperature (20.0°C). Near the end of the 5 minute exposure time, the dried films were scraped with a cell scraper and at the end of the exposure time the virus-test substance mixtures were immediately passed through prepared individual Sephadex columns utilizing the syringe plungers to detoxify the mixtures. The filtrates (10^{-1} dilution) were then titered by 10-fold serial dilution. The Hep-2 cells in multiwell culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions from the test and control groups and were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 11 days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus film recovery, cytotoxicity, neutralization and cell viability.
Note: Protocol deviations/amendments reported in this study were reviewed and found to be acceptable.

15. MRID 490895-20 "Aqualogic Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Adenovirus type 5", for product Aqualogic, Lot Numbers 051512DT-3 and 052912DT-1. Study Director Lisa Hellickson. Study conducted by Ecolab. Study completion date, Dec 18, 2012. Study ID Number 1200059.

This study was conducted using two lots of Aqualogic Lots 051512DT-3 (batch 3) and 052912DT-1 (batch 1), which were tested according to Ecolab Microbiological Services SOP Method; *Virucidal Efficacy Assay for Hard Surfaces* to determine the Virucidal efficacy against Adenovirus type 5, strain Adenoid 75 (ATCC VR-5) with a 5% fetal bovine serum organic soil load, after 5 minutes exposure at ambient temperature. Testing followed GLP Protocol Study No. 1200059 (copy provided). The two lots of test substance were diluted to 660 ppm free available chlorine in sterile laboratory purified water or Milli-Q water. Lot 051512DT-3 dilution 246.96 g test substance plus 103.05 g diluent and Lot 052912DT-1 dilution 253.75 g test substance plus 96.26 g diluent to achieve 660 ppm available chlorine. HeLa Cells (ATCC CCL-

2), from the third or greater transfer, were prepared in 24 well assay plates and were incubated at 35±2°C in a humidified atmosphere at 5±2% CO_2 for two days prior to the test. Test medium used to maintain the cell cultures was Minimum Essential Medium, Eagle (EMEM) test medium, supplemented with 10% (v/v) heat inactivated fetal bovine serum and 50-100 IU penicillin and 50-100 µg/mL streptomycin. Several vials of stock Adenovirus type 5 were thawed and pooled on the day of the test and since 5% Fetal Bovine Serum (FBS) is present in the virus stock, no additional soil load was added. The bottom of 100 x 15 mm glass Petri dishes were inoculated with 0.2 mL of prepared virus/soil mixture and dried for 37 minutes in a biological safety cabinet. Each batch of use-solution was dispensed into one glass Petri dish with a dried virus film by spraying at a distance of 6 to 8 inches using 3 trigger pulls at ambient (15°-30°C) temperature. Near the end of the 5 minute exposure time, the dried film was scraped from the surface of the dish with a cell scraper, and 0.1 mL of the virus/test mixture was pipetted into prepared sephacryl columns (0.1 mL per column – five columns used per batch) for neutralization. The columns were centrifuged for 2 minutes at 700 x g after which the supernatant was serially diluted and inoculated into assay plates seeded with the test cell culture (0.1 mL per well). Four assay wells were inoculated per dilution prepared and were incubated at 35±2°C in a humidified atmosphere at 5±2% CO_2 for 7-10 days. Controls included those for dried virus film recovery, virus stock titer confirmation, cytotoxicity, neutralization and cell viability.
Note: Protocol deviations/amendments reported in this study were reviewed and found to be acceptable.

16. MRID 490895-21 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces", Virus: Human Coronavirus, for product Aqualogic, Batch 073112-CL1 and Batch 080112-CL1. Study Director Mary J. Miller, M.T., Study completion date, Sept. 21, 2012, Study conducted by ATS Labs. Project Number A13926.

This study was conducted using two batches of test substance "Aqualogic", Batch 073112-CL1 and Batch 080112-CL1, which were tested against Human Coronavirus, ATCC VR-740, Strain 229E with a 5% fetal bovine serum organic soil load. Testing followed Protocol Number ECO01072512.COR (copy provided). The host cell line, WI-38 (human lung) cells, ATCC CCL-75, were seeded into multiwell cell culture plates and maintained and used in tissue culture at 36-38°C in a humidified atmosphere at 5-7% CO_2. Test medium used to maintain the cell cultures was Minimum Essential Medium (MEM) supplemented with 2% (v/v) heat-activated fetal bovine serum (FBS), 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. The two batches of test substance were diluted to the lower limit of 660 ppm available chlorine in sterile deionized water. Batch 073112-CL1 dilution 357.38 g test substance plus 142.62 g diluent and Batch 080112-CL1 dilution 352.41 g test substance plus 147.59 g diluent to achieve 660 ppm available chlorine. On the day of use, an aliquot of stock virus, was thawed and adjusted to contain 5% FBS as the organic soil load. The bottoms of three separate 100 x 15 mm glass Petri dishes were inoculated with 0.2 mL of prepared virus/soil mixture and dried at 20.0°C at 50% relative humidity for 20 minutes (until visibly dry). Each batch of test substance was dispensed into one glass Petri dish with a dried virus film by spraying at a distance of 6 to 8 inches using 3 trigger pulls at room temperature (20.0°C). Near the end of the 5 minute exposure time, the dried films were scraped with a cell scraper and at the end of the exposure time the virus-test substance mixtures were immediately passed through prepared individual Sephadex columns utilizing the syringe plungers to detoxify the mixtures. The filtrates (10^{-1} dilution) were then titered by 10-fold serial dilution. The Hep-2 cells in multiwell culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions from the test and control groups and were incubated at 31-35°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 11 days for the absence or presence of CPE, cytotoxicity, and for

viability. Controls included those for dried virus film recovery, cytotoxicity, neutralization and cell viability.
Note: Protocol deviations/amendments reported in this study were reviewed and found to be acceptable.

17. MRID 490895-22 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces", Virus: Murine Norovirus, for product Aqualogic, Batch 073112-CL1 and Batch 080112-CL1. Study Director Shanen Conway, B.S., Study completion date, Sept. 21, 2012. Study conducted by ATS Labs. Project Number A13932.

This study was conducted using two batches of test substance "Aqualogic", Batch 073112-CL1 and Batch 080112-CL1, which were tested against Murine Norovirus (MNV-1.CW1 Strain), obtained from Washington University, St. Louis, MO. Testing followed Protocol Number ECO01072512.MNV (copy provided). The host cell line, RAW 264.7 cells, a continuous mouse macrophage cell line was seeded into multiwell cell culture plates maintained and used in tissue culture at 36-38°C in a humidified atmosphere at 5-7% CO_2. Test medium used to maintain the cell cultures was Complete 2X MEM. The two batches of test substance were diluted to the lower limit of 660 ppm available chlorine in sterile deionized water. Batch 073112-CL1 dilution 356.99 g test substance plus 143.01 g diluent and Batch 080112-CL1 dilution 352.78 g test substance plus 147.22 g diluent to achieve 660 ppm available chlorine. On the day of use, an aliquot of stock virus was thawed and adjusted to contain 5% FBS as the organic soil load. The bottoms of three separate 100 x 15 mm glass Petri dishes were inoculated with 0.2 mL of prepared virus/soil mixture and dried at 20.0°C at 50% relative humidity for 20 minutes (until visibly dry). Each batch of test substance was dispensed into one glass Petri dish with a dried virus film by spraying at a distance of 6 to 8 inches using 3 trigger pulls at room temperature (20.0°C). Near the end of the 30 seconds exposure time, the dried films were scraped with a cell scraper and at the end of the exposure time the virus-test substance mixtures were immediately passed through prepared individual Sephadex columns utilizing the syringe plungers to detoxify the mixtures. The filtrates (10^{-1} dilution) were then titered by 10-fold serial dilution. The RAW 264.7 macrophage cells in multiwell culture dishes were inoculated in quadruplicate with 250 µL aliquot of the dilutions from the test and control groups. Following adsorption of 60 minutes, the inoculum was removed and an aliquot of MNV Overlay Agarose I was inoculated into each well, which were then incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 for 2 days. Following incubation, an aliquot of MNV Overlay Agarose I with neutral red stain was added and the cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 for 4 hours after which the cultures were microscopically observed to verify plaques or test substance cytotoxicity. Controls included those for dried virus film recovery, cytotoxicity, neutralization and cell viability.
Note: Protocol deviations/amendments reported in this study were reviewed and found to be acceptable.

18. MRID 490895-23 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Duck Hepatitis B Virus as a Surrogate Virus for Human Hepatitis B Virus", for product Aqualogic, Batch 073112-CL1 and Batch 080112-CL1. Study Director Shanen Conway, B.S., Study completion date, Oct. 15, 2012. Study conducted by ATS Labs. Project Number A13931.

This study was conducted using two batches of test substance "Aqualogic", Batch 073112-CL1 and Batch 080112-CL1, which were tested against Duck Hepatitis B Virus as a Surrogate Virus for Human Hepatitis B Virus (10/29/11 strain obtained from Hepadnavirus Testing Inc). The

stock virus contained 100% duck serum as the organic soil load. Testing followed Protocol Number ECO01072512.DHBV (copy provided). The host cell line, hepatocytes obtained from blood samples from Pekin hatchling ducks less than 7 days old, were seeded into sterile 12 well tissue culture labware. The cultures were maintained and used at the appropriate density and incubated at 36-38°C in a humidified atmosphere at 5-7% CO_2. Test medium used to maintain the cell cultures was Leibovitz L-15 medium supplemented with 10 µM dexamethasone, 10 µg/mL insulin, 20 mM HEPES, 10 µg/mL gentamicin, and 100 units/mL penicillin The two batches of test substance were diluted to the lower limit of 660 ppm available chlorine in sterile deionized water. Batch 073112-CL1 dilution 356.99 g test substance plus 143.01 g diluent and Batch 080112-CL1 dilution 352.78 g test substance plus 147.22 g diluent to achieve 660 ppm available chlorine. On the day use, two aliquots of stock virus (Lot 10/29/11 pool) was thawed, combined and refrigerated until use. The stock virus cultures contained 100% duck serum as the organic soil load. The bottoms of three 100 x 15 mm glass petri dishes were inoculated with 0.2 mL of prepared virus/soil mixture and dried at 20.0°C at 50% relative humidity for 30 minutes (until visibly dry). The carriers were sprayed at a distance of 6 to 8 inches using 3 trigger pulls and were exposed for 5 minutes at room temperature (20.0°C). Near the end of the exposure time, the dried films were individually scraped with a cell scraper, and at the end of the exposure time the virus-test substance mixtures were immediately passed through prepared individual Sephadex columns utilizing the syringe plungers to detoxify the mixtures. The filtrates (10^{-1} dilution) were then titered by 10-fold serial dilution. The primary duck hepatocyte cells in cell culture dishes were inoculated in quadruplicate with 250 µL of the dilutions from the test and control groups and were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 for 9 days. The test medium was aspirated from each test and control well and replaced with fresh medium as needed throughout the incubation period. On the final day of incubation, the cultures were scored microscopically for cytotoxicity and the cells were fixed with ethanol. An indirect immunofluorescence assay was then performed using a monoclonal antibody specific for the envelope protein of the DHBV.
Note: Protocol deviations/amendments reported in this study were reviewed and found to be acceptable.

19. MRID 490895-24 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Virus: Herpes simplex virus type 2" for the product Aqualogic. Study director is Shanen Conway. Study conducted by ATS Labs. Study completion date – September 26, 2012. Project Number A13929.

This study was conducted against the G strain of Herpes simplex virus type 2 (ATCC VR-734) with rabbit kidney (RK) cells as the host cell line. RK cells were obtained from ViroMed Laboratories, Inc. Two lots of Aqualogic (073112-CL1 and 080112-CL1) were tested according to ATS protocol ECO01080812.HSV2 (copy provided). The Aqualogic test substance was diluted to the lower certified limit in sterile deionized water to yield 660 ppm available chlorine as follows:

Lot 073112-CL1; 357.38g + 142.62 g diluent = 660 ppm available chlorine
Lot 080112-CL1; 352.41g + 147.59 g diluent = 660 ppm available chlorine

The stock virus was prepared by collecting the supernatant culture fluid from 75-100% infected culture cells and stored at <-70°C until the day of use. On the day of use, an aliquot of stock virus (ATS Labs Lot H2-64) was removed, thawed, and maintained refrigerated. The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 200 µL of virus inoculum uniformly over the bottoms of three separate 100 x 15 mm sterile glass Petri dishes. The virus films were dried at 20.0°C in a relative humidity of 50% until visibly dry (20 minutes). For each lot of test substance, one dried virus film was individually exposed for 30 seconds at room temperature (21.0°C) to the amount of spray released under

use conditions. The carriers were sprayed 3 sprays, until thoroughly wet at a distance of 6-8 inches and held covered for the exposure time. The virus films were completely covered with the test substance. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to resuspend the contents and at the end of the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates (10^{-1} dilution) were then titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity in RK cells. RK cells in multi-well culture dishes were inoculated in quadruplicate with 100 µL of the dilutions prepared from test and control groups. All cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 in sterile disposable cell culture labware. The host RK cells were examined microscopically and scored periodically for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability for seven days. Controls included those for plate recovery, cytotoxicity, neutralization and cell viability. Viral and Cytotoxicity titers were calculated by the Spearman Kaber method.
Note: Protocol deviations/amendments reported in this study were reviewed and found to be acceptable.

20. MRID 490895-25 "Aqualogic Germicidal Spray Disinfection Efficacy Against Antibiotic Resistant Organisms" for the product Aqualogic. Study director is Laurinda Holen. Study conducted by Ecolab. Study completion date – January 11, 2013. Project Number 1200073.

This study was conducted against;
Acinetobacter baumannii (MDR ATCC BAA-1605),
Staphylococcus aureus (VISA ATCC 700788),
Staphylococcus aureus (CA-MRSA ATCC BAA-1683),
Staphylococcus aureus (MRSA ATCC 33592),
Klebsiella pneumoniae (carbapenemase producer (KPC) ATCC BAA-1705), and *Enterococcus faecalis* (VRE ATCC 51299).

Two lots (051512DT and 052912DT) of the product, Aqualogic, were tested using Ecolab Microbiological Services SOP Method MS010-21 (copy provided). The Aqualogic test substance was diluted to the lower certified limit in sterile laboratory purified water to yield 660 ppm available chlorine as follows:

Lot 51512DT-2-UD1; 499.26g + 200.74g diluent = 660 ppm available chlorine
Lot 52912DT-2-UD1; 513.12g + 186.87g diluent = 660 ppm available chlorine
Lot 51512DT-2-UD2; 359.72g + 140.26g diluent = 660 ppm available chlorine
Lot 52912DT-2-UD2; 369.39g + 130.62g diluent = 660 ppm available chlorine
Lot 51512DT-2-UD4; 501.97g + 198.05g diluent = 660 ppm available chlorine
Lot 52912DT-2-UD4; 583.92g + 116.08g diluent = 660 ppm available chlorine

As per protocol, the test organisms were prepared by inoculation from stock cultures and transferred greater than 3 times but less than or equal to 15 times in a suitable growth medium prior to being used in the test. An appropriate number of tubes with 20 mL culture media were inoculated with each organism and incubated at 35 ± 2°C for 48-54 hours prior to testing. The culture media was AOAC nutrient broth for the all of the test organisms with a supplement of antibiotics added for *A. baumannii* and *S. aureus* VISA only. The test cultures were vortexed and allowed to settle for 15 minutes. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Twenty six (26) non-corrosive microscope coverslips (1"x1") were used as carriers and were inoculated with 0.01 mL of each test organism. The inoculum was spread uniformly over the carrier and placed in a sterile Petri dish matted with 2 layers of Whatman No. 2 filter paper. The Petri dishes were covered and placed in a 35 ± 2°C incubator for 30 minutes

to dry. The inoculated coverslips were sprayed with 3 sprays of the test substance at a distance of approximately 6-8 inches. After the 5 minute exposure period, the excess test substance was allowed to drain off the carrier prior to aseptically transferring to individual 32 x 200 mm test tubes containing 20 mL of appropriate neutralizer/subculture medium using sterile forceps. Neutralization medium was Letheen broth + 0.5% sodium thiosulfate for test organisms except for *S. aureus* MRSA which was D/E broth. The subculture agar for all test organisms was Tryptone Glucose Extract. All subculture tubes and agar plates were incubated at 35 ± 2°C for 48 ± 4 hours. Following incubation, the subcultures were examined for growth. Controls included those for confirmation of antibiotic resistance by test organisms, purity, sterility, viability, neutralization confirmation, bacteriostasis, and carrier population. Antibiotic susceptibility testing was performed on *Acinetobacter baumannii* (MDR ATCC BAA-1605), *Staphylococcus aureus* (CA-MRSA ATCC BAA-1683), *Staphylococcus aureus* (MRSA ATCC 33592), and *Enterococcus faecalis* (VRE ATCC 51299) using the disk diffusion method to confirm antibiotic resistance as described in Ecolab's procedures MS111-04 or MS111-05 (Antibiotic Susceptibility Tests). Multi drug resistance was verified for *Acinetobacter baumannii* (MDR ATCC BAA-1605) using 120 mcg gentamicin disks, 10 µg imipenem disks, and 30 meg ceftazidime disks. Methicillin resistance was verified for *Staphylococcus aureus* (CA-MRSA ATCC BAA-1683) and *Staphylococcus aureus* (MRSA ATCC 33592) using 1 meg oxacillin disks. Vancomycin resistance was verified for *Enterococcus faecalis* (VRE ATCC 51299) using 30 µg vancomycin disks. Antibiotic susceptibility testing was performed on *Staphylococcus aureus* (VISA ATCC 700788) using the minimum inhibitory concentration (MIC) method to confirm intermediate resistance to vancomycin. Carbapenemase production was verified for *Klebsiella pneumoniae* (carbapenemase producer (KPC) ATCC BAA-1705) by using the Modified Hodge Test.
Note: Protocol deviations/amendments reported in this study were reviewed and found to be acceptable.

21. MRID 490895-26 "Aqualogic Germicidal Spray Disinfection Efficacy at 10 Minutes" for the product Aqualogic. Study director is Laurinda Holen. Study conducted by Ecolab. Study completion date – January 11, 2013. Project Number 1200077.

This study was conducted against *Enterococcus faecalis* (VRE ATCC 51299) and *Escherichia coli* O157:H7 (ATCC 43895). Two lots (051512DT and 052912DT) of the product, Aqualogic, were tested using Ecolab Microbiological Services SOP Method MS010-21 (copy provided). The Aqualogic test substance was diluted to the lower certified limit in sterile laboratory purified water to yield 660 ppm available chlorine as follows:

Lot 51512DT-3-UD; 357.78g + 142.23g diluent = 660 ppm available chlorine
Lot 52912DT-3-UD; 380.48g + 119.51g diluent = 660 ppm available chlorine
Lot 51512DT-3-UD2; 286.52g + 113.46g diluent = 660 ppm available chlorine
Lot 52912DT-3-UD2; 311.58g + 88.41g diluent = 660 ppm available chlorine

As per protocol, the test organisms were prepared by inoculation from stock cultures and transferred greater than 3 times but less than or equal to 15 times in a suitable growth medium prior to being used in the test. An appropriate number of tubes with 20 mL culture media were inoculated with each organism and incubated at 35 ± 2°C for 48-54 hours prior to testing. The culture media was brain heart infusion broth for *E. coli* and brain heart infusion broth plus 100 uL 400 µg/mL vancomycin per 10 mL broth for *E. faecalis*. The test cultures were vortexed and allowed to settle for 15 minutes. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Twenty six (26) non-corrosive microscope coverslips (1"x1") were used as carriers and were inoculated with 0.01 mL of each test organism. The inoculum was spread uniformly over the carrier and placed in a sterile Petri dish matted with 2 layers of Whatman No.

2 filter paper. The Petri dishes were covered and placed in a 35 ± 2°C incubator for 30 minutes to dry. The inoculated coverslips were sprayed with 3 sprays of the test substance at a distance of approximately 6-8 inches. After the 10 minute exposure period, the excess test substance was allowed to drain off the carrier prior to aseptically transferring to individual 32 x 200 mm test tubes containing 20 mL of appropriate neutralizer/subculture medium using sterile forceps. Neutralization medium was brain heart infusion broth + 0.5% sodium thiosulfate for *E. coli* with the addition of 0.7 g lecithin per liter and 5.0 g Tween 80 per liter for *E. faecalis*. The subculture agar for both test organisms was brain heart infusion agar. All subculture tubes and agar plates were incubated at 35 ± 2°C for 48 ± 4 hours. Following incubation, the subcultures were examined for growth. Controls included those for confirmation of antibiotic resistance by test organism, purity, sterility, viability, neutralization confirmation, bacteriostasis, and carrier population. Vancomycin resistance was verified for *Enterococcus faecalis* (VRE ATCC 51299) using 30 µg vancomycin disks and the disk diffusion method.
Note: Protocol deviations/amendments reported in this study were reviewed and found to be acceptable.

22. MRID 490895-27 "Aqualogic Germicidal Spray Disinfection Efficacy with *Trichophyton mentagrophytes* and *Aspergillus niger*" for the product Aqualogic. Study director is Laurinda Holen. Study conducted by Ecolab. Study completion date – January 24, 2013. Project Number 1200058.

This study was conducted against *Trichophyton mentagrophytes* (ATCC 9533) and *Aspergillus niger* (ATCC 6275). Two lots (051512DT and 052912DT) of the product, Aqualogic, were tested using Ecolab Microbiological Services SOP Method MS010-21 (copy provided). The Aqualogic test substance was diluted to the lower certified limit in sterile laboratory purified water to yield 660 ppm available chlorine as follows:

Lot 51512DT-1-UD; 352.41g + 147.59g diluent = 660 ppm available chlorine
Lot 52912DT-1-UD; 359.72g + 140.29g diluent = 660 ppm available chlorine
Lot 51512DT-3-UD5; 288.12g + 111.92g diluent = 660 ppm available chlorine
Lot 52912DT-3-UD5; 346.39g + 53.59g diluent = 660 ppm available chlorine
Lot 51512DT-3-UD7; 292.56g + 107.42g diluent = 660 ppm available chlorine
Lot 52912DT-1-UD7; 368.16g + 31.86g diluent = 660 ppm available chlorine

The test organisms were transferred from Sabouraud Dextrose Agar slants to Sabouraud Dextrose agar and incubated at 26 + 2°C for 10-15 days prior to inoculating a second set of plates which were incubating at 26 ± 2°C for an additional 10-15 days. After incubation, the mycelial mats were removed from the surface of the agar by adding 5 mL of 0.85% saline to each plate. The culture suspension was transferred to a sterile glass tissue grinder and macerated with additional 0.85% saline. The suspension was then filtered through two layers of sterile cheesecloth to remove the hyphal elements. The density of the suspension was estimated by performing serial dilutions and plating on Sabouraud Dextrose agar. 0.02 mL of Triton X-100 was added per 10 mL of the test organism suspensions to facilitate spreading onto the glass slide carrier and fetal bovine serum was added to suspensions to achieve 5% organic soil load. Thirteen to twenty six (26) non-corrosive microscope coverslips (1"x1") were used as carriers and were inoculated with 0.01 mL of each test organism suspension. The inoculum was spread uniformly over the carrier and placed in a sterile Petri dish matted with 2 layers of Whatman No. 2 filter paper. The Petri dishes were covered and placed in a 35 ± 2°C incubator for 30 minutes to dry. The inoculated coverslips were sprayed with 3 sprays of the test substance at a distance of approximately 6-8 inches. After the 5 or 10 minute exposure period, the excess test substance was allowed to drain off the carrier prior to aseptically transferring to individual 32 x 200 mm test tubes containing 20 mL of appropriate neutralizer/subculture medium using sterile forceps. The neutralization/ subculture medium for *A. niger* was

Sabouraud Dextrose Broth + 0.5% Sodium Thiosulfate and for *T. mentagrophytes* was Sabouraud Dextrose Broth + 0.7 g Lecithin/L + 5.0 g Tween 80/L. The subculture agar for both test organisms was Sabouraud Dextrose agar. All subculture tubes and agar plates were incubated at 26 ± 2°C for 3-5 days. Following incubation, the subcultures were examined for growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

23. MRID 490895-28 "Aqualogic Germicidal Spray Disinfection Efficacy" for the product Aqualogic. Study director is Laurinda Holen. Study conducted by Ecolab. Study completion date – January 24, 2013. Project Number 1200072.

This study was conducted against;

Acinetobacter baumannii (ATCC 19606),
Escherichia coli (ATCC 11229),
Klebsiella pneumoniae (ATCC 4352),
Serratia marcescens (ATCC 14756),
Shigella flexneri (ATCC 9380),
Enterobacter aerogenes (ATCC 13048),
Escherichia coli O157:H7 (ATCC 43895),
Streptococcus pyogenes (ATCC 19615),
Shigella dysenteriae (ATCC 29026),
Listeria monocytogenes (ATCC 7644), and
Enterococcus faecalis (ATCC 29212).

Two lots (051512DT and 052912DT) of the product, Aqualogic, were tested using Ecolab Microbiological Services SOP Method MS010-20 and MS010-21 (copies provided). The Aqualogic test substance was diluted to the lower certified limit in sterile laboratory purified water to yield 660 ppm available chlorine as follows:

Lot 51512DT-1-UD1; 354.57g + 145.45g diluent = 660 ppm available chlorine
Lot 52912DT-1-UD1; 362.78g + 137.23g diluent = 660 ppm available chlorine
Lot 51512DT-1-UD2; 496.01g + 203.98g diluent = 660 ppm available chlorine
Lot 52912DT-1-UD2; 508.58g + 191.40g diluent = 660 ppm available chlorine
Lot 51512DT-2-UD1; 499.79g + 200.22g diluent = 660 ppm available chlorine
Lot 52912DT-2-UD1; 503.60g + 196.39g diluent = 660 ppm available chlorine
Lot 51512DT-3-UD4; 501.97g + 198.05g diluent = 660 ppm available chlorine
Lot 52912DT-2-UD4; 583.92g + 116.08g diluent = 660 ppm available chlorine
Lot 51512DT-3-UD7; 292.56g + 107.42g diluent = 660 ppm available chlorine
Lot 52912DT-1-UD7; 368.16g + 31.86g diluent = 660 ppm available chlorine

As per protocol, the test organisms were prepared by inoculation from stock cultures and transferred greater than 3 times but less than or equal to 15 times in a suitable growth medium prior to being used in the test. An appropriate number of tubes with 20 mL culture media were inoculated with each organism and incubated at 35 ± 2°C for 48-54 hours prior to testing with the exception of *Serratia marcescens* which was incubated at 26 ± 2°C for 48-54 hours and *Enterobacter aerogenes* which was incubated at 30 ± 2°C for 48-54 hours. The culture media was AOAC nutrient broth for the all of the test organisms except for *Escherichia coli* O157:H7, *Streptococcus pyogenes*, *Listeria monocytogenes*, and *Enterococcus faecalis* which were grown in brain heart infusion broth. The test cultures were vortexed and allowed to settle for 15 minutes. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Thirteen to twenty six (26) non-corrosive microscope coverslips (1"x1") were used as carriers and were inoculated with 0.01 mL of each test organism. The inoculum was spread uniformly over the carrier and placed in a sterile Petri dish matted with 2 layers of Whatman No. 2 filter paper. The Petri dishes were covered and placed in a 35 ± 2°C incubator for 30-33 minutes to

dry. The inoculated coverslips were sprayed with 3 sprays of the test substance at a distance of approximately 6-8 inches. After the 5 minute exposure period, the excess test substance was allowed to drain off the carrier prior to aseptically transferring to individual 32 x 200 mm test tubes containing 20 mL of appropriate neutralizer/subculture medium using sterile forceps. Neutralization medium was Letheen broth + 0.5% sodium thiosulfate for test organisms except for; *Escherichia coli* O157:H7 which was brain heart infusion broth + 0.5% Sodium Thiosulfate; *Streptococcus pyogenes*, *Listeria monocytogenes*, and *Enterococcus faecalis* was D/E broth. The subculture agar for all test organisms was Tryptone Glucose Extract except for *Escherichia coli* O157:H7, *Streptococcus pyogenes*, *Listeria monocytogenes*, and *Enterococcus faecalis* which was brain heart infusion agar. All subculture tubes and agar plates were incubated at 35 ± 2°C for 48 ± 4 hours with the exception of *Serratia marcescens* which was incubated at 26 ± 2°C for 48 ± 4 hours and *Enterobacter aerogenes* which was incubated at 30 ± 2°C for 48 ± 4 hours. Following incubation, the subcultures were examined for growth. Controls included those for purity, sterility, viability, neutralization confirmation, bacteriostasis, and carrier population.
Note: Protocol deviations/amendments reported in this study were reviewed and found to be acceptable.

V. RESULTS

Table 1. UDM Test Results 5-Minute Exposure Time at 660 ppm Chlorine

MRID Number	Organism	No. Carriers Exhibiting Growth/ Total No. Carriers Tested			Average Carrier Population (CFU/carrier)
		Lot 51512DT-1	Lot 52912DT-1	Lot 50112DT-1*	
490895-12	*Staphylococcus aureus* (ATCC 6538)	1/60	0/60	0/60	3.7×10^6
	Pseudomonas aeruginosa (ATCC 15442)	0/60	0/60	0/60	4.5×10^5
	Salmonella enterica (ATCC 10708)	1/60	0/60	1/60	5.0×10^5

*≥60 days old

MRID Number	Organism	Lot	No. Exhibiting Growth/Total No. Tested	Carrier Population (CFU/carrier)
5-Minute Exposure Time at 660 ppm Chlorine				
490895-25	*Acinetobacter baumannii* (MDR ATCC BAA-1605)	51512DT-2-UD1	0/10	4.6×10^4
		52912DT-2-UD1	0/10	
	Staphylococcus aureus (CA-MRSA ATCC BAA-1683)	51512DT-2-UD1	0/10	3.8×10^5
		52912DT-2-UD4	0/10	
	Klebsiella pneumoniae (KPC) (ATCC BAA-1705)	51512DT-2-UD2	0/10	1.2×10^5
		52912DT-2-UD2	0/10	
	Staphylococcus aureus (VISA ATCC 700788)	51512DT-2-UD2	0/10	8.3×10^5
		52912DT-2-UD2	0/10	
	Staphylococcus aureus (MRSA ATCC 33592)	51512DT-3-UD4	0/10	1.1×10^6
		52912DT-2-UD4	0/10	

MRID Number	Organism	Lot	No. Exhibiting Growth/Total No. Tested	Carrier Population (CFU/carrier)
5-Minute Exposure Time at 660 ppm Chlorine				
490895-28	*Acinetobacter baumannii* (ATCC 19606)	51512DT-1-UD1	0/10	1.0×10^6
		52912DT-1-UD1	0/10	
	Escherichia coli (ATCC 11229)	51512DT-1-UD1	0/10	4.6×10^4
		52912DT-2-UD1	0/10	
	Serratia marcescens (ATCC 14756)	51512DT-1-UD1	0/10	4.8×10^6
		52912DT-1-UD1	0/10	
	Klebsiella pneumoniae (ATCC 4352)	51512DT-1-UD1	0/10	2.9×10^5
		52912DT-1-UD1	0/10	
	Escherichia coli O157:H7 (ATCC 43895)	51512DT-1-UD2	3/10	3.0×10^6
		52912DT-1-UD2	0/10	
	Enterobacter aerogenes (ATCC 13048)	51512DT-1-UD2	0/10	7.7×10^5
		52912DT-2-UD4	0/10	
	Shigella flexneri (ATCC 9380)	51512DT-2-UD1	0/10	3.0×10^4
		52912DT-2-UD1	0/10	
	Shigella dysenteriae (ATCC 29026)	51512DT-2-UD1	0/10	3.2×10^6
		52912DT-2-UD1	0/10	
	Listeria monocytogenes (ATCC 7644)	51512DT-3-UD4	0/10	3.1×10^5
		52912DT-2-UD4	0/10	
	Enterococcus faecalis (ATCC 29212)	51512DT-3-UD4	0/10	8.8×10^5
		52912DT-2-UD4	0/10	
	Streptococcus pyogenes (ATCC 19615)	51512DT-3-UD7	0/10	5.0×10^4
		52912DT-1-UD7	0/10	

MRID Number	Organism	Lot	No. Exhibiting Growth/Total No. Tested	Carrier Population (CFU/carrier)
10-Minute Exposure Time at 660 ppm Chlorine				
490895-26	*Escherichia coli* O157:H7 (ATCC 43895)	51512DT-3-UD	0/10	2.4×10^6
		52912DT-3-UD	0/10	
	Enterococcus faecalis (VRE ATCC 51299)	51512DT-3-UD2	0/10	2.1×10^6
		52912DT-3-UD2	0/10	

MRID Number	Organism	Lot	No. Exhibiting Growth/Total No. Tested	Carrier Population (CFU/carrier)
5-Minute Exposure Time at 660 ppm Chlorine				
490895-27	*Aspergillus niger* (ATCC 6275)	51512DT-1-UD	3/10	2.2×10^6
		52912DT-1-UD	0/10	
	Trichophyton mentagrophytes (ATCC 9533)	51512DT-3-UD5	2/10	2.8×10^5
		52912DT-3-UD5	0/10	
10-Minute Exposure Time at 660 ppm Chlorine				
490895-27	*Aspergillus niger* (ATCC 6275)	51512DT-3-UD5	0/10	1.3×10^4
	Trichophyton mentagrophytes (ATCC 9533)	51512DT-3-UD7	0/10	1.9×10^5
		52912DT-1-UD7	0/10	

MRID Number	Organism	Lot No.	Average No. Surviving CFU/mL	Microbes Initially Present CFU/mL	Percent Reduction
3-Minute Exposure Time at 260 ppm Chlorine					
490895-13	*Enterobacter aerogenes* (ATCC 13048)	51512DT-1	$<2.5 \times 10^{1}$	1.4×10^{9}	>99.9
		52912DT-2	$<2.5 \times 10^{1}$	9.9×10^{8}	>99.9
		50112DT-1*	$<2.5 \times 10^{1}$	5.3×10^{8}	>99.9
4-Minute Exposure Time at 260 ppm Chlorine					
490895-13	*Staphylococcus aureus* (ATCC 6538)	51512DT-2	$<6.3 \times 10^{2}$	3.5×10^{8}	>99.9
		52912DT-2	$<1.8 \times 10^{3}$	2.8×10^{8}	>99.9
		50112DT-1*	$<1.6 \times 10^{2}$	1.6×10^{8}	>99.9

*≥60 Days old

MRID Number	Organism	Lot No.	Average No. Surviving CFU/mL	Microbes Initially Present CFU/mL	Percent Reduction
1-Minute Exposure Time at 660 ppm Chlorine					
490895-14	*Enterobacter aerogenes* (ATCC 13048)	51512DT-1	$<2.5 \times 10^{1}$	1.4×10^{9}	>99.9
		52912DT-2	$<2.5 \times 10^{1}$	9.9×10^{8}	>99.9
		50112DT-1*	$<2.5 \times 10^{1}$	5.3×10^{8}	>99.9
	Staphylococcus aureus (ATCC 6538)	51512DT-1	$<3.6 \times 10^{2}$	2.6×10^{8}	>99.9
		52912DT-2	$<8.1 \times 10^{2}$	2.8×10^{8}	>99.9
		50112DT-1*	$<6.1 \times 10^{1}$	1.6×10^{8}	>99.9

*≥60 Days old

MRID Number	Organism	Results @ 30 Second Contact Time 660 ppm Chlorine	Lot # 073112-CL1	Lot # 080112-CL1	Dried Virus Count
490895-16	HIV type 1 (strain HTLV-III_B)	10^{-1} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{5.50}$ $TCID_{50}$/0.2 mL
		$TCID_{50}$/0.2 mL	$\leq 10^{1.50}$	$\leq 10^{1.50}$	
490895-22	Murine Norovirus (MNV-1.CW1 Strain)	10^{-1} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	$10^{6.25}$ PFU_{50}/250 µL
		PFU_{50}/250 µL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
490895-24	Herpes Simplex Virus Type 2 (HSV2) (ATCC VR-734)	10^{-1} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{5.25}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	

MRID Number	Organism	Results @ 30 Second Contact Time 660 ppm Chlorine	Lot # 051512DT-2	Lot # 052912DT-2	Dried Virus Count
490895-07	Rhinovirus Type 37 (strain 151-1 ATCC VR-1147)	10^{-1} to 10^{-5} dilutions	Complete inactivation	Complete inactivation	$10^{4.50}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	
490895-10	Herpes Simplex Type 1 (strain F (1) ATCC VR-733)	10^{-1} to 10^{-5} dilutions	Complete inactivation	Complete inactivation	$10^{4.50}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	

MRID Number	Organism	Results @ 30 Second Contact Time 660 ppm Chlorine*			Dried Virus Count**
			Lot # 051512DT-2	Lot # 052912DT-1	
490895-08	Norovirus [Feline Calcivirus (strain F-9 ATCC VR-782) as surrogate]	10^{-1} to 10^{-5} dilutions	Complete inactivation	Complete inactivation	$10^{6.25}$
		$TCID_{50}$/0.1 mL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	$TCID_{50}$/0.1 mL

*Both Lots had two replicates. Lot 0515112DT-2 was originally tested as Lot 051512DT-3, but one replicate showed CPE in an abnormal pattern. Testing on this lot was repeated twice to verity that CPE was due to aerosolizaton. The final assessment was complete inactivation as shown above.
**Six different dried virus tests were conducted and all passed. Only one is presented here.

MRID Number	Organism	Results @30 Second Contact Time 660 ppm Chlorine			Dried Virus Count
			Lot # 051512DT-2	Lot # 052912DT-3	
490895-15	Influenza A Virus (H1N1 strain A/Virginia/ATCC1/2009 ATCC VR-1736)	10^{-1} to 10^{-5} dilutions	Complete Inactivation	Complete Inactivation	$10^{4.25}$
		$TCID_{50}$/0.1mL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	$TCID_{50}$/0.1mL

MRID Number	Organism	Results @30 Second Contact Time 260 ppm Chlorine			Dried Virus Count
			Replicate 1 Lot # 051512DT-2	Replicate 1 Lot # 052912DT-2	
490895-09	Norovirus [Feline Calcivirus (strain F-9 ATCC VR-782) as surrogate]	10^{-1} to 10^{-5} dilutions	Complete Inactivation	Complete Inactivation	$10^{6.25}$
		$TCID_{50}$/0.1mL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	$TCID_{50}$/0.1mL
			Replicate 2 Lot # 051512DT-2	Replicate 2 Lot # 052912DT-2	
		10^{-1} to 10^{-5} dilutions	Complete Inactivation	Complete Inactivation	$10^{6.50}$
		$TCID_{50}$/0.1mL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	$TCID_{50}$/0.1mL
490895-06	Rhinovirus Type 37 (strain 151-1 ATCC VR-1147)	10^{-1} to 10^{-5} dilutions	Complete Inactivation	Complete Inactivation	$10^{4.25}$
		$TCID_{50}$/0.1mL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	$TCID_{50}$/0.1mL

MRID Number	Organism	Results @30 Second Contact Time 260 ppm Chlorine			Dried Virus Count
			Lot # 051512DT-2	Lot # 052912DT-3	
490895-11	Influenza A Virus (H1N1 strain A/Virginia/ATCC1/2009 ATCC VR-1736)	10^{-1} to 10^{-5} dilutions	Complete Inactivation	Complete Inactivation	$10^{4.38}$
		$TCID_{50}$/0.1mL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	$TCID_{50}$/0.1mL

MRID Number	Organism	Results @5 Minute Contact Time 660 ppm Chlorine			Dried Virus Count
			Lot # 051512DT-3	Lot # 052912DT-1	
490895-20	Adenovirus type 5, strain Adenoid 75 (ATCC VR-5)	10^{-1} to 10^{-5} dilutions	Complete Inactivation	Complete Inactivation	$10^{6.50}$
		$TCID_{50}$/0.1mL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	$TCID_{50}$/0.1mL

MRID Number	Organism	Results @ 5 Minute Contact Time 660 ppm Chlorine			Dried Virus Count
			Lot # 073112-CL1	Lot # 080112-CL1	
490895-17	Rotavirus, Strain WA	10^{-1} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	$10^{5.50}$ $10^{6.25**}$
		$TCID_{50}$/0.1 mL	$\leq 10^{0.50*}$	$\leq 10^{0.50}$	$TCID_{50}$/0.1 mL
490895-18	Vaccina virus, Strain WR (ATCC VR-119)	10^{-1} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	$10^{7.50}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
490895-19	Respiratory syncytial (RSV) virus (ATCC VR-26, Strain Long)	10^{-1} to 10^{-6} dilutions	Complete inactivation	Complete inactivation	$10^{5.00}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
490895-21	Human Coronavirus, ATCC VR-740, Strain 229E	10^{-1} to 10^{-6} dilutions	Complete inactivation	Complete inactivation	$10^{4.75}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
490895-23	Duck Hepatitis B Virus as a Surrogate Virus for Human Hepatitis B Virus	10^{-1} to 10^{-4} dilutions	Complete inactivation	Complete inactivation	$TCID_{50}$/0.250 mL $10^{5.75}$
		$TCID_{50}$/0.250 mL	Replicate 1 $\leq 10^{0.50}$	Replicate 1 $\leq 10^{0.50}$	
			Replicate 2 $\leq 10^{0.50}$	Replicate 2 $\leq 10^{0.50}$	$TCID_{50}$/0.250 mL $10^{6.00}$

*Tested twice (8/24/12 and 10/04/12)
**Result for second test for Lot 073112-CL1

VI. CONCLUSIONS

1.) The submitted efficacy data **support** the use of Hydris at *a* dilution of 660 ppm chlorine in sterile deionized water as a disinfectant against the following organisms on hard, non-porous surfaces in the presence of *a* 5% organic soil load for a 5-minute contact time:

Staphylococcus aureus (ATCC 6538)	MRID 490895-12
Pseudomonas aeruginosa (ATCC 15442)	MRID 490895-12
Salmonella enterica (ATCC 10708)	MRID 490895-12
Acinetobacter baumannii (MDR ATCC BAA-1605)	MRID 490895-25
Staphylococcus aureus (VISA ATCC 700788)	MRID 490895-25
Staphylococcus aureus (CA-MRSA ATCC BAA-1683)	MRID 490895-25
Staphylococcus aureus (MRSA ATCC 33592)	MRID 490895-25
Klebsiella pneumoniae (KPC) ATCC BAA-1705)	MRID 490895-25
Acinetobacter baumannii (ATCC 19606)	MRID 490895-28
Escherichia coli (ATCC 11229)	MRID 490895-28
Klebsiella pneumoniae (ATCC 4352)	MRID 490895-28
Serratia marcescens (ATCC 14756)	MRID 490895-28

Shigella flexneri (ATCC 9380)	MRID 490895-28
Enterobacter aerogenes (ATCC 13048)	MRID 490895-28
Streptococcus pyogenes (ATCC 19615)	MRID 490895-28
Shigella dysenteriae (ATCC 29026)	MRID 490895-28
Listeria monocytogenes (ATCC 7644)	MRID 490895-28
Enterococcus faecalis (ATCC 29212)	MRID 490895-28

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth. Antibiotic resistance was demonstrated for resistant test organisms in confirmation tests. Neutralization confirmation testing showed positive growth of the microorganisms.

2.) The submitted efficacy data **support** the use of Hydris at a dilution of 660 ppm chlorine in sterile deionized water as a disinfectant against the following organisms on hard, non-porous surfaces in the presence of a 5% organic soil load for a 10-minute contact time:

Escherichia coli O157:H7 (ATCC 43895)	MRID 490895-26
Enterococcus faecalis (VRE ATCC 51299)	MRID 490895-26
Aspergillus niger (ATCC 6275)	MRID 490895-27
Trichophyton mentagrophytes (ATCC 9533)	MRID 490895-27

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth. Neutralization confirmation testing showed positive growth of the microorganisms. Antibiotic resistance was demonstrated for *Enterococcus faecalis* (VRE) in confirmation tests.

3.) The submitted efficacy data **support** the use of Hydris at a dilution of 260 ppm chlorine in 250 ppm sterile hard water as a sanitizer for inanimate, non-food contact surfaces against the following bacteria in the presence of 5% organic soil load for 3-minute contact at room temperature for *Enterobacter aerogenes* (ATCC 13048) and 4 minute contact at *Staphylococcus aureus* (ATCC 6538).

Enterobacter aerogenes (ATCC 13048)	MRID 490895-13
Staphylococcus aureus (ATCC 6538)	MRID 490895-13

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

4.) The submitted efficacy data **support** the use of Hydris at a dilution of 660 ppm chlorine in sterile deionized water as a sanitizer for inanimate, non-food contact surfaces against the following bacteria in the presence of 5% organic soil load for a 1-minute contact at room temperature.

Enterobacter aerogenes (ATCC 13048)	MRID 490895-14
Staphylococcus aureus (ATCC 6538)	MRID 490895-14

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Viability controls were positive for growth. Purity

controls were reported as pure. Sterility controls did not show growth.

5.) The submitted efficacy data **support** the use of Hydris at a dilution of 260 ppm chlorine as a disinfectant with virucidal activity against the following microorganisms on hard, non-porous surfaces in the presence of a 5% organic soil load for and sterile 400 ppm hard water for a 30 second contact time:

Rhinovirus Type 37 -260 ppm (strain 151-1 ATCC VR-1147)	MRID 490895-06
Feline Calcivirus (strain F-9 ATCC VR-782)	MRID 490895-09
Influenza A Virus (H1N1 strain A/Virginia/ATCC1/2009 ATCC VR-1736)	MRID 490895-11

Recoverable virus titers of at least 10^4 were achieved. No cytotoxicity was observed. Complete inactivation (no growth) was indicated in all dilutions tested.

6.) The submitted efficacy data **support** the use of Hydris at a dilution of 660 ppm chlorine as a disinfectant with virucidal activity against the following microorganisms on hard, non-porous surfaces in the presence of a 5% organic soil load for a 30 second contact time:

Rhinovirus Type 37 (strain 151-1 ATCC VR-1147)	MRID 490895-07
Norovirus [Feline Calcivirus (strain F-9 ATCC VR-782) as surrogate]	MRID 490895-08
Herpes Simplex Type 1 (strain F (1) ATCC VR-733)	MRID 490895-10
Influenza A Virus (H1N1 strain A/Virginia/ATCC1/2009 ATCC VR-1736)	MRID 490895-15
HIV type 1 (strain HTLV-III_B)	MRID 490895-16
Murine Norovirus (MNV-1.CW1 Strain)	MRID 490895-22
Herpes Simplex Virus Type 2 (HSV2) (ATCC VR-734)	MRID 490895-24

Recoverable virus titers of at least 10^4 were achieved. Cytotoxicity was only observed with HIV type 1 (strain HTLV-III_B) and only at a dilution of 10^{-1}. Complete inactivation (no growth) was indicated in all other dilutions tested.

7.) The submitted efficacy data **support** the use of Hydris at a dilution of 660 ppm chlorine as a disinfectant with virucidal activity against the following microorganisms on hard, non-porous surfaces in the presence of a 5% organic soil load for a 5 minute contact time:

Rotavirus, Strain WA	MRID 490895-17
Vaccina virus, Strain WR (ATCC VR-119)	MRID 490895-18
Respiratory syncytial (RSV) virus (ATCC VR-26, Strain Long)	MRID 490895-19
Adenovirus type 5, strain Adenoid 75 (ATCC VR-5)	MRID 490895-20
Human Coronavirus, ATCC VR-740, Strain 229E	MRID 490895-21
Duck Hepatitis B Virus as a Surrogate Virus for Human Hepatitis B Virus	MRID 490895-23

Recoverable virus titers of at least 10^4 were achieved. Cytotoxicity was not observed. Complete inactivation (no growth) was indicated in all dilutions tested.

VII. RECOMMENDATIONS

1. The label claims that Hydris™ (825 ppm FAC) is an effective disinfectant against the following organisms on hard, non-porous surfaces for a 5-minute contact time:

Acinetobacter baumannii (MDR ATCC BAA-1605),
Staphylococcus aureus (VISA ATCC 700788),
Staphylococcus aureus (CA-MRSA ATCC BAA-1683),
Staphylococcus aureus (MRSA ATCC 33592),
Klebsiella pneumoniae (carbapenemase producer (KPC) ATCC BAA-1705),
Acinetobacter baumannii (ATCC 19606),
Escherichia coli (ATCC 11229),
Klebsiella pneumoniae (ATCC 4352),
Serratia marcescens (ATCC 14756),
Shigella flexneri (ATCC 9380),
Enterobacter aerogenes (ATCC 13048),
Streptococcus pyogenes (ATCC 19615),
Shigella dysenteriae (ATCC 29026),
Listeria monocytogenes (ATCC 7644)
Enterococcus faecalis (ATCC 29212),
Staphylococcus aureus (ATCC 6538),
Pseudomonas aeruginosa (ATCC 15442), and
Salmonella enterica (ATCC 10708).

These claims are acceptable as they are supported by the submitted data.

2. The label claims that Hydris™ (825 ppm FAC) is an effective disinfectant against the following organisms on hard, non-porous surfaces for a 10-minute contact time:

Escherichia coli O157:H7 (ATCC 43895)
Enterococcus faecalis (VRE ATCC 51299)
Aspergillus niger (ATCC 6275)
Trichophyton mentagrophytes (ATCC 9533)

These claims are acceptable as they are supported by the submitted data.

3. The label claims that Hydris™ (260 ppm FAC) in 250 ppm hard water is an effective non-food contact sanitizer against the following organisms on hard, non-porous surfaces for a 4-minute contact time:

Staphylococcus aureus (ATCC 6538)
Enterobacter aerogenes (ATCC 13048)

These claims are acceptable as they are supported by the submitted data.

4. The label claims that Hydris™ (825 ppm FAC) is an effective non-food contact sanitizer against the following organisms on hard, non-porous surfaces for a 1-minute contact time:

Staphylococcus aureus (ATCC 6538)
Enterobacter aerogenes (ATCC 13048)

These claims are acceptable as they are supported by the submitted data.

5. The label claims that Hydris™ (260 ppm FAC) is an effective virucide against the following organisms on hard, non-porous surfaces in the presence of a 5% organic soil load and 400 ppm hard water for a 30-second contact time:

Rhinovirus Type 37 (strain 151-1 ATCC VR-1147)
Norovirus [Feline Calcivirus (strain F-9 ATCC VR-782) as surrogate]
Influenza A Virus (H1N1 strain A/Virginia/ATCC1/2009
ATCC VR-1736)

These claims are acceptable as they are supported by the submitted data.

6. The label claims that Hydris™ (825 ppm FAC) is an effective virucide against the following organisms on hard, non-porous surfaces in the presence of a 5% organic soil load for a 30-second contact time:

Rhinovirus Type 37 (strain 151-1 ATCC VR-1147)
Norovirus [Feline Calcivirus (strain F-9 ATCC VR-782) as surrogate]
Herpes Simplex Type 1 (strain F (1) ATCC VR-733)
Influenza A Virus (H1N1 strain A/Virginia/ATCC1/2009 ATCC VR-1736)
HIV type 1 (strain $HTLV\text{-}III_B$)
Murine Norovirus (MNV-1.CW1 Strain)
Herpes Simplex Virus Type 2 (HSV2) (ATCC VR-734)

These claims are acceptable as they are supported by the submitted data.

7. The label claims that Hydris™ (825 ppm FAC) is an effective virucide against the following organisms on hard, non-porous surfaces in the presence of a 5% organic soil load for a 5 minute contact time:

Rotavirus, Strain WA
Vaccina virus, Strain WR (ATCC VR-119)
Respiratory syncytial (RSV) virus (ATCC VR-26, Strain Long)
Adenovirus type 5, strain Adenoid 75 (ATCC VR-5)
Human Coronavirus, ATCC VR-740, Strain 229E
Duck Hepatitis B Virus as a Surrogate Virus for
Human Hepatitis B Virus

These claims are acceptable as they are supported by the submitted data.

LABEL RECOMMENDATIONS

- The label should state that the ready to use product is 825 ppm FAC and provide dilution instructions where the label states to use a lower FAC for sanitizing and some virucide uses.
- The statement "This product has demonstrated effectiveness against influenza A virus and is expected to inactivate all Influenza A viruses including Pandemic 2009 H1N1 influenza A virus" is too broad and needs modification as not all strains have been tested.
- Statements such as "kills 99.9% of bacteria" and "germicidal" need to refer to the labeled organisms.